Reply

1) CONTENT OF THE WRITTEN OPINION

The written opinion from the International Examining Authority, indicates that the present invention is easily obtained on the basis of the cited reference 1 (TAJIMA Hideji "Automation for separation and isolation of nuclei acid" the bulletin of Japanese Applied Magnetics (1998, May) No.22, 5th,p1010-1015), cited reference 2 (Japanese Laid-Open Publication No.9-28397. Dade International Inc.) and cited reference 3 (Japanese Laid-Open Publication No.6-343496, Hitachi Ltd.), cannot be noticed to have unexpected remarkable merits and does not meet the non-obviousness requirement. We reply this written opinion below.

2) EXPLANATION OF THE PRESENT INVENTION AND THE CITED REFERENCES

As shown in amended claim 1 of the Claims, the present invention is a labeled complex comprising, a carrier, a large number of target receptors bonded with said carrier, and labeled substances bonded with each target receptor at different locations from a location at which said carrier is bonded, wherein said target receptor holds or can hold one or two or more types of targets between said two locations, and in all of said labeled substances, two or more predetermined types are contained at predetermined molar ratios.

The claim 1 is amended on the basis that the gist of the present invention is to enable the discrimination of more than thousands or tens of thousands of types of targets (as described on page 3, lines 19 to 24, for example). Mutual discrimination between those targets is not possible by using only the existing types of labeled substances, but requires two more types of labeled substances that are contained at predetermined molar ratios.

Hence, the claim 1 is amended so as to be limited to the case of using “two more types”.

The amendment for insertion of "between said two locations" is derived from drawings and embodiments, for example.

Furthermore, the claim 32 is amended for clearing that the labeled complex of the claim 32 is that of any one of claims 1 to 11.

The Claim 35 is added as an independent claim on the basis of the description "suspension suspending group of the labeled complex" of the Claim 22.

With regard to the cited reference 1, there is a description, "desired Nuclei-acid is simultaneously separated from a plenty of samples at a time by bonding specific reaction substances such as various antibody, primer, prove or the like to surface of magnetic particles", on lines 12 to 15 of the left column on page 1011 of the cited reference 1, as the Examiner indicated.

With regard to the cited references 2, there is a description "A process to determine the presence or concentration of an analyte, wherein fluorescent magnetic particles are used to monitor the number of particles present during said process.", in the claim 1 of the cited reference 2. Further, with the processes, there is a description, "contacting fluorescent magnetic particles having a ligand specific for said analyte attached to said fluorescent magnetic particles". Namely, "contacting fluorescent magnetic particles having a ligand specific for said analyte attached to said fluorescent magnetic particle with fluid specimen to form a suspension, separating said magnetic particles from said suspension, adding a second labeled ligand specific for said analyte to said separated magnetic particles, separating said magnetic particles from said suspension, detecting or measuring duplex formation on said magnetic particles by means of said label, and relating the amount of labeled ligand measured with the amount of analyte measured for

a control sample."

Further, with the fluorescent magnetic particles, in claim 6 of the cited reference 2, there is the description, "an inner fluorescent core polymer particle able to adsorb a monomer and a magnetically responsive metal oxide and polymer combination said polymer being comprised of monomers able to adsorb to said inner core polymer particles and holding a fluorescent material or, a mixture of one or more of the fluorescent materials, and said metal oxide and polymer combination evenly coating said inner core particle".

Besides, in the claim 2 of the cited reference 2, there is the description, "fluorescent material is selected from the group consisting of various material or a mixture of one or more of these fluorescent materials". Furthermore, in the claim 3, there is " monitoring the number of said fluorescent magnetic particles by measuring fluorescent intensity".

In a paragraph 【0006】 of the cited reference 2, there is a passage, " Spectrum characteristic can be changed by using core particles in which including various fluorescent dyes consisting of one or more of the fluorescent dyes.". In a paragraph【0007】 of the cited reference 2, there is a passage, "The fluorescent intensity can be regulated by varying content of magnetically responsive metal oxide for changing shade by the metal oxide and/or by changing amount of fluorescent dyes including in the core particles".

In Fig. 1 of the cited reference 2, the fluorescent magnetic particles which comprises a fluorescent core particle 1, metal oxide/ polymer coating layer2 and polymer protective coating layer 3 covering the core particle 1, and polymer layer 4 with functional group for bonding various materials and covering the layer 2, 3, are disclosed.

The cited reference 3 disclosed, in Fig. 1 etc. a method comprising the

following steps: dispensing DNA prove 402 bonding with labeled micro-particles 401, DNA prove 404 bonding with micro-particles 403 whose diameter are larger than that of the micro-particles 401, forming two strand by hybridizing with sample DNA 405, thereafter removing non-connected remnant DNA prove 402 by using a filter whose diameter of pore has that between the two kinds of micro particles, cutting off the two strand by restriction enzyme 407, and separating fluorescent micro-particles 401 free from the micro-particles 403 by a filter 406, and calculating number of the particles by introducing to the flow cell.

3) COMPARISON BETWEEN THE PRESENT INVENTION AND TECHNOLOGIES OF THE CITED REFERENCES

The present invention of claim 1 seems to be similar to technologies of the cited references 1, 2, 3, in that those use carrier and labeled substances such as fluorescent material and so on and targets are carried by the carrier.

However, the construction and purpose of the present invention are different from those of the cited references and the present invention has various outstanding merits that the cited references do not disclose, as shown below.

Though in the cited reference 1, there is a description "simultaneously separating desired nuclei acid from a plenty of sample, at a time by bonding specific reaction substances such as various antibodies, primers, probes to the magnetic particles", the cited reference 1 fails to disclose the construction of the magnetic particles, target receptor and the labeled substances as well as mutual discrimination of plenty of types of targets by using various predetermined types of labeled substances being contained at predetermined molar ratios.

With the cited reference 2, as shown in Fig. 1 etc, the fluorescent

magnetic particle comprises the labeled substances layer including fluorescent dyes which covers the surface of the inner core polymer particle(carrier), and polymer layer with functional group for bonding targets and covering the labeled substances layer. With the cited reference 2, when a plenty of targets bond to the polymer layer with functional group of this fluorescent magnetic particle, the target shades the fluorescent substances optically.

In contrast thereto, the labeled complex of the present invention has such construction that the labeled substances are supported not by the carrier itself, but by a plenty of target receptors bonded with carrier. Further, the target receptor bonds with the carrier at a location thereof, and bonds with the labeled substance at the different locations from the location. Between the two locations, there is a target itself, target is held or target can be held. Therefore, the labeled complex is different from the fluorescent magnetic particles, in that the labeled substance does not cover over the surface of the carrier directly and the targets do not cover the labeled substances directly and does not shade optically.

According to the difference of the construction, with the present invention, existence of the target or the target receptor and existence of the labeled substances mutually do not affect bad influence respectively. Namely, increase of the number of target receptor does not cause decrease of the whole amount of the labeled substances and does not reduce labeling ability.

Or oppositely increase of the whole amount of labeled substances does not cause decrease of the whole number of the target receptor and does not reduce holding ability of the carrier. Rather, increase of the number of target receptor causes increase of amount of the labeled substances. Since holding ability of the target and labeling ability cooperate mutually, the present invention has such effect that the more increases the number of the

target receptor and quantity of the labeled substances, the more accurate and reliable inspection results can be obtained.

With the cited reference 2, core particles and metal-oxide layer need preliminarily be processed so that they are covered with fluorescent substance layer or the protective layer and need preliminarily be processed so that the fluorescent layer and the protective layer are covered with polymer with functional group. Hence, the process requires much expense in cost and effort.

In contrast thereto, the present invention needs only process for bonding the target receptor with the carrier. Since the other process can be executed by reaction process in suspension, the present invention has such effect that the process requires less cost and manpower.

Furthermore, with the cited reference 2, since the polymer with functional group is formed so as to cover the fluorescent layer and the protection layer, the types of the polymer are limited to the types which can bond with the fluorescent layer or the protection layer, and the diversity is restricted.

In contrast thereto, with the present invention, since the condition of the material for carrier is not strict, chemically and physically stable materials can be selected. Therefore, various process such as covering with various materials can be executed for bonding various target receptor. Consequently, the present invention has high diversity and applicability, because of being able to process for various targets.

Furthermore, with regard to the present invention, the labeled substances do not directly bond with the carrier in such a manner that they densely occupy the surface thereof, but bond with the carriers through the target receptor. Therefore, the longest length between labeled substances bonded with neighboring target receptor equals to the sum of length along

the target receptor between location at which the labeled substances bonds with and location at which the carrier bonds with. Therefore, such affect by interaction between different types of labeled substance (for example, quenching) can be avoided, and clear detection can be executed.

Further, the present invention uses two or more predetermined types of labeled substances being contained at predetermined molar ratios per carrier, for enabling to discriminate various types of targets simultaneously. In contrast thereto, the cited reference 2 is still in such a stage to only adjust the spectrum intensity by using a single labeled substance or combination of labeled substances in the fluorescent layer, or a single fluorescent dyes or combination of fluorescent dyes in an inner core polymer particle, in which molar ratios are not determined.

Though, various types of labeled substances are exemplified in the claim 2 of the cited reference 2, one of the labeled substances or one selected from the mixture of the labeled substances is used. The cited reference 2 does not disclose or suggest such purpose or construction that two or more labeled substances in one carrier are used for mutual discrimination of the carrier. Really, the purpose of the cited reference 2 is to measure whether the target exists or not, or the density. Hence, with regard to the cited reference 2, it is essential only that one type of target can be labeled. Because of the difference of construction and purpose, the present invention has such an effect that plenty of types of targets that can not be discriminated by using the number of types of labeled substances or the number of mixture of types of labeled substances, can be clearly discriminated.

The purpose of method disclosed in the cited reference 3, is to measure the density of the target in the sample for extracting peculiar substances bonded to target from the same type of substances, as mentioned above. As

it were, the technology of the cited reference 3 corresponds to the method which uses one type of labeled substances for discriminating one type of target and equals to the case of the cited reference 2. The cited reference 3 does not disclose the discrimination of various types of targets or carriers by bonding two or more predetermined types of labeled substances being contained at predetermined quantity ratios with each carrier, as shown in the present invention.

Consequently, the present invention has various effects that the cited reference 3 as well as cited reference 2, does not discloses.

Though only claim 1 of this application is replied above, the other claims are also replied. Because, the claims 2 to 11 cite claim 1, the claims 12 to 19 are method for producing the labeled complex of claim 1, the claims 20 to 31 are method for using the labeled complex of claim 1, claims 32 to 34 are an analysis device for measuring the quantity ratios of the labeled complex of claim 1, and claim 35 is a suspension suspended by the labeled complex of claim 1. Therefore, the construction and purpose of the other claims are also different from those of the cited references 1,2,3, from the above mentioned reasons, and the other claims have various effects that the cited references have not.

4) CONCLUSION

As mentioned above, the cited reference 1 discloses general description in regard to labeling. The cited references 2, 3 disclose the labeled substances are used only for discriminating the labeled particles from non-labeled particles. Consequently, all the cited references 1, 2, 3 do not disclose such a technical thought that discriminate between a plenty of types of carriers or targets by using two or more types of the labeled substances being contained at predetermined molar ratios.

Thus, since all the cited references 1, 2, 3 do not disclose the above technical thought, even if the cited references 1, 2, 3 are collected, the present invention can not be obtained. Furthermore, with the present invention, since the labeled substances are bonded with the carry through the target receptor which holds or can hold targets. Therefore, as mentioned above, the present invention has the following various outstanding merits:

Quenching which occur between different types of labeled substances, can be avoided, the labeled substances are not optically be sheltered by targets, the ability for holding target is not reduced, manpower for processing is reduced, and a plenty of types of targets whose number are more larger than that of types of labeled substances or that of combination of the types thereof, can be discriminated.

In spite of having the above mentioned various merits, those points are not disclosed or suggested in these cited references. Consequently, the present invention meet mot only the novelty requirements, but also non-obviousness requirement, on the basis of the cited references.

Consequently, the applicant requests to issue the International Preliminary Examination Reports stating that the present invention has novelty and non-obviousness.

6. Amendments

(1) On pages44, line 6, "of targets, and in all of said labeled substances, predetermined types are" is replaced with "of targets between said two locations, and in all of said labeled substances, two or more predetermined types are".

(2) On page 50, line 20, "A target analyzing apparatus which utilizes a labeled complex, said" are replaced with "A target analyzing apparatus which utilizes a labeled complex according to any one of claims 1 to 11, said".

(3) New claim 35 is added to the CLAIMS.

7. LIST OF ATTACHED DOCUMENTS: pages 44, 50 and 51 of Claims

CLAIMS:

1. (AMENDED) A labeled complex comprising, a carrier, a large number of target receptors bonded with said carrier, and labeled substances bonded with each target receptor at different locations from a location at which said carrier is bonded, wherein said target receptor holds or can hold one or two or more types of targets between said two locations, and in all of said labeled substances, two or more predetermined types are contained at predetermined molar ratios.

2. A labeled complex according to claim 1, wherein all of said labeled substances are distributed to almost all target receptors bonded with one carrier, and one target receptor is bonded with one type of labeled substance.

3. A labeled complex according to either one of claim 1 and claim 2, wherein said target receptor, which is bonded with the carrier on a part thereof, and bonded with the labeled substance on the other part thereof, is formed in a slender shape such as a line, a thread, a hair, a stick and the like.

4. A labeled complex according to any one of claim 1 through claim 3, wherein said target receptors comprise chemical compounds which contain biopolymers such as nucleic acids, peptides, proteins, polysaccharides, lipids and the like, or living beings such as viruses, bacteria and the like or a part thereof, or substances which hold or are able to hold them.

5. A labeled complex according to any one of claim 1 through claim 4, wherein said target receptors comprise nucleic acids having a predetermined double strand base sequence, said labeled substance is bonded with only a single strand at one location, and said carrier is bonded with the other single strand in at another location.

6. A labeled complex according to any one of claim 1 through claim 4, wherein said target receptor comprises nucleic acid having a predetermined double strand base sequence, said labeled substance is bonded only with one

location of a single strand, and said carrier is fixed to another location of the

30. A process for utilizing a labeled complex according to either one of claim 20 and claim 21, wherein said selection step has; a step for labeling said labeled complex by luminescent substances, for labeling the selective substances by different types of luminescent substances from the labeled substances, and for mixing and contacting the liquid in which the labeled complex group is suspended with the selective substances, and a step for passing suspended liquid of the labeled complex group including labeled complexes bonded with the selective substances through a translucent narrow tube, and said discrimination step has a step for receiving light when the suspended liquid of said labeled complex group passes through said narrow tube, and a step wherein, with respect to the labeled complex selected by the measurement of the intensity of light emitted by the selective substance, based on the result of a measurement of the intensity of light emitted by the labeled complex, the types and the molar ratio are computed to discriminate the corresponding target.

31. A process for utilizing a labeled complex according to either one of claim 21, wherein in the case where said discrimination substances or selective substances are fluorescent substances or mineral phosphates, in the step for passing said suspended liquid through said narrow tube, an excitation light for exciting the substances is emitted toward said narrow tube.

32. (AMENDED) A target analyzing apparatus which utilizes a labeled complex according to any one of claims 1 to 11, said apparatus having a transfer pump for transferring liquid within which a labeled complex group is suspended, a translucent narrow tube through which the suspension passes, light detecting means for detecting light from the discrimination substances and selective substances of the labeled complex when passing through the narrow tube, and analyzing means for analyzing the intensity of light received by said light detecting means, selecting the labeled complex, and discriminating a target.

33. A target analyzing apparatus which utilizes a labeled complex according to claim 32, wherein there is further provided irradiating means for externally radiating excitation light toward said narrow tube for, in the case where said discrimination substances or selective substances are fluorescent substances or mineral phosphates, exciting the substances, or providing light for scattering to obtain scattered light from the substances.

34. A target analyzing apparatus which utilizes a labeled complex according to claim 32, wherein said narrow tube forms part of a closed circuit, and the suspension of said labeled complex group is circulated in the closed circuit.

35. (ADDED) A suspension incorporating group of labeled complex comprising, a carrier, a large number of target receptors bonded with said carrier, and labeled substances bonded with each target receptor at different locations from a location at which said carrier is bonded, wherein said target receptor holds or can hold one or two or more types of targets, and in all of said labeled substances,

predetermined types are contained at predetermined molar ratios.